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PHASES IN THE LIFE HISTORY OF A HOLOSTOME, *CYATHOCOTYLE ORIENTALIS* NOV. SPEC.,

WITH NOTES ON THE EXCRETORY SYSTEM OF THE LARVA *

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Adult holostomes have been known for many years, while the tetracotyliform larvae have been figured in many of the early works on helminths. Since the investigation of Ercolani on *Tetracotyle typica* (1881) the genetic relationship of larva to adult has been understood, but the remainder of the life cycle has been in doubt. Most helminthologists have regarded the life history as monogenetic, although adequate evidence on this question has not been advanced. I have shown in a previous paper (Faust 1918: 15) that in one species, *Tetracotyle flabelliformis,* the larval holostome arises parthenogenetically within a redia, supporting the view of digenetic development. Moreover, the embryo of *Strigea (Holostomum) cornucopiae* figured by von Linstow (1877) and Brandes (1890) is, without doubt, a miracidium, so that the only part of the life cycle lacking experimental proof in one or another holostome species is that bridging the gap between the miracidium and redia.

Furthermore, the homologies of the holostome group have been comprehended by few investigators, mostly because they have not traced fundamental structures from larva to adult. However, Odhner has shown a keen insight into the relationship of the group and to him belongs the distinction of clearing up many doubtful points.

It is hoped that the study I have recently had an opportunity to pursue will serve to make the group of the holostomes better understood along certain lines.

Tetracotyliform larvae were obtained during the spring of 1921 from the testes of the lesser edible snail, *Vivipara lapillorum* Heude, secured from the North Lake of the Imperial City, Peking, and from the Erh Châ Canal outside the southeast corner of the Tartar City. In all nearly seven hundred snails were collected of which 10.3 per cent. harbored these holostome larvae. The same hosts were also infected to a lesser degree with a cystocercous cercaria, *C. pekinensis,* echinostome cercariae, xiphidocercariae, monostome cercariae, a cercarieum species and aspidobothrids. All of the holostomes obtained

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were in the encysted condition. In some instances only a few were obtained from each infected host; in other cases several hundred were secured. In one host the liver gland as well as the testis was infected.

Description of *Tetracotyle orientalis* nov. spec.

The encysted tetracotyle measures 0.4 to 0.5 mm. in length by 0.3 to 0.4 mm. in transverse diameter. It is pyriform and is covered with minute spines over the anterior half of the body. I have not been able to dissect away the cyst wall without injuring the larva, but the internal structure has been readily studied through the capsule when the specimens were compressed under a cover glass.

In the uncompressed condition the animal is very active, moving freely within the cyst wall. On the ventral side there are, in addition to the oral sucker, several conspicuous structures typical of the tetracotyle. The oral sphincter measures about 45 μ in diameter. Somewhat posterior to it is a pair of lateral suctorial grooves, which consist only of auricular depressions in the body and lack true sphincter muscles. Slightly posterior to these is a large pouch extending to the subdistal region of the body. In longitudinally contracted specimens the anterior margin of the pouch canopy all but forms a straight line at right angles to the long axis of the worm. At other times its lateral ends almost meet anteriorly, constituting the broken circumference of a circle. Just under this canopy on the floor of the pouch in the midline lies the functional ventral sucker, subequal to the oral sucker. Behind it lies the ventral suctorial apparatus (Fig. 1), consisting of a divided anterior lip and a single posterior lip. I have shown for another species, *Tetracotyle iturbei* (Faust 1919: 71), that the functional ventral sucker is the primitive genital pore, whereas the posterior ventral suctorial apparatus is the homolog of the true ventral sucker of distomes. While the present study reveals no such striking homology, the anterior and posterior ventral suctorial structures may be regarded in the same light.

Internally, the digestive apparatus and the excretory system have been studied in living material while the beginnings of the genital organs have been observed in stained mounts. Within the oral sucker there is a small pharynx, 15μ in transverse diameter. Behind this is a short well-defined esophagus. The ceca are simple and extend backward in a broad bow only as far as the ventral suctorial apparatus.

The genital organs are retarded in their development. In many tetracotyliform larvae from mollusk hosts the organs can be readily differentiated. In this species only the vitellaria have become separated from the germinal cells and have come to occupy a position lateral to the lateral suctorial grooves. The remaining organs still lie in the

genital complex posterior to the ventral suctorial apparatus, with a longitudinal cord of cells extending to the posterior margin of the worm.

The Excretory System in the Larva

The excretory system of the holostome larva consists of two parts, the bladder and the tubule system with the flame-cells at the termination of the capillaries. Odhner (1913: 312) correctly described the former (the reserve bladder) as a close subcutaneous network of relatively wide canals. These are usually recognized by the granules which occupy the canal system and which are pushed back and forth along the canals by the movements of the living worm. Each described species has a fairly unique pattern of such granules, although those of *Tetracotyle flabelliformis* and of the species here described show several points of resemblance (Fig. 4, right half). In many of the described tetracotyles it has been impossible to distinguish between the granular-canal system (the reserve bladder) and the capillary-tubule system which constitutes the primary excretory apparatus. The latter is seemingly very complex, albeit mathematically exact, and is frequently concealed by the superficial network of granules. The few flame-cells which can be seen at times in most forms give no true conception of the real pattern of the system while an attempt to trace them out leads one to see the difficulties in the study.

In the case of *Tetracotyle orientalis,* there was abundant material for study which was constantly available through more than two months of the spring. This enabled me to select only those specimens for study of the flame-cells which contained a minimum amount of granules, but even then the apparent complexity of the flame-cells required many hours of patient work to explain. But when the system was actually unfolded its real simplicity and the precision of the divarications were immediately evident (Fig. 4, left side).

The bladder *(sensu stricto)* consists of a short median portion with a pair of cornua. From the anterior end of each cornu there arises a primary tubule which extends anteriad close to the lateral margin of the worm. Five secondary tubules are given off at graduated distances along the primary tubule. Each of these latter subdivides into equal dorsal and ventral portions. Each portion, too, has a five-fold dichotomy. This provides the dorsal and ventral portions of each side of the body with 160 flame cells, whereas each one of the five main tubules drains 64 flame cells. The entire system, therefore, consists of 640 flame-cells.

Since the system when fully known is so readily reduced to a simple formula, I suggest that it be designated as $\alpha + \beta + \gamma + \delta + \epsilon$, corresponding to the symbols used for the basic excretory apparatus in my previous papers. While further subdivision of the capillaries

and flame cells may occur as the larva grows into the adult, studies on the excretory system of trematodes have now arrived at a point that one may predict with reasonable assurance that, in such instances, the sum will be a multiple of the least common denominator, in this case, $\alpha + \beta + \gamma + \delta + \epsilon$.

Furthermore, it is evident from studies I have made of many living specimens of this and other species that the network of granules is only a reserve system, which is extremely useful in the encysted larva when the capsule prevents ejection of the excretory material, but which is not in use in the free-living post-cystic condition.

Experimental Data on the Life History

During my study of the holstome larva, I attempted feeding experiments on young Chinese domestic ducks with the idea of testing out whether these larvae could continue growth in this type of host.

Altogether, 6 young ducks were obtained. They had never been near any ponds or canals and had been fed only on a mash consisting of cooked millet and cooked cabbage leaves. They had been raised by hand in a mat basket. When about one month old they were brought to the laboratory. They were fed daily on a similar diet of cooked millet and cooked cabbage leaves. For a period of ten days they were observed and their feces examined daily for ova. On April 1, 1921 one of the ducklings, at that time one month old, was infected *per orem* with about 200 encysted *Tetracotyle orientalis* and a few encysted echinostomula. Four of the other ducks were infected with other larval trematodes, while one was kept as a check. The diet of millet and cabbage was continued, and the feces examined daily. On the twelfth day large trematode eggs were recovered from the feces of the duck experimentally fed with holostome larvae, while the other animals remained negative. The positive animal remained in good health until the seventeenth day, when it developed a case of acute diarrhoea and died within a few hours. Examination of the diarrhoeic stool showed several of the same large eggs, while the autopsy of the animal showed several immature echinostomes in the small intestine and seventy-five small holostomes in the ceca. The holostomes were closely applied to the cecal mucosa which was blood streaked, while the lumen was filled with a purulent fluid.

It is my belief that the worms were responsible in part for the death of the host. All the other ducks were negative up to the time of their death which occurred after that of the positive animal and on autopsy showed no infection.

Examination of the holostomes recovered showed that about half of them were mature, with one or two eggs in the uterus, while the others were less advanced. A study of the adult specimens has proved

them to belong to the genus Cyathocotyle Mühling 1896. They cannot be allocated to the described species of this genus, and for them I suggest the name *Cyathocotyle orientalis*.

Description of *Cyathocotyle orientalis* nov. spec.—The mature worm is pyriform and measures from 0.7 to 1.3 mm. in length by 0.5 to 0.9 mm. in width (Fig. 3). The body is covered with small spines in the anterior half. The lateral view has the appearance of a short cucumber, except for the large suctorial cup on the ventral side. The worm is readily recognized as a holostome because the genital ducts open into a posteriorly located genital atrium. *Cyathocotyle orientalis* has an oral sucker which is 110 μ in diameter. It leads directly into a pharynx 45 μ in transsection. From it there proceeds a short but unmistakable esophagus such as Odhner (1913: 312) has shown to obtain for this group. The ceca constitute a graceful bow which extends to the subdistal region of the body. They consist of large epithelial cells with glandular contents.

The most noticeable feature of the mature worm is the genital system, and of this system the two testes are the most conspicuous elements. They consist of a smaller oval body ($350 \times 230 \mu$) situated anterior and sinistral to the mid region of the worm, and a larger similar body ($480 \times 270 \mu$) to the right and posterior to the midregion of the worm (Fig. 3, *T*). A pair of short slender vasa efferentia arise from them and proceed to the very short vas deferens which opens immediately into the coiled vesicula seminalis. This merges with the large cirrus sac, which contains a long penial organ. The latter may be protruded as much as 100 μ outside of the genital chamber. The vesicula seminalis is filled with spermatozoa. No prostate glands have been observed.

The ovary is a small ovoid body lying anterior to the right testis. It opens by a short oviduct, which joins Laurer's canal as it proceeds to the ootype. There is a receptaculum seminis which exists as a lateral outpocketing of Laurer's canal for the storage of spermatozoa. In this respect I agree with Mühling (quoted by Odhner, 1913: 309) in finding a seminal receptacle and must, therefore, differ from Odhner himself who regards the contents as similar to the flame-cilia of the fertilization-room of the oviduct. The contents are long, coiled and stain similarly to the contents of the vesicula seminalis. The vitellaria of the adult develop from the two masses of finely divided materials differentiated as vitelline glands in the tetracotyle. In the adult, however, they are aggregated into discrete glands packed with vitelline material. A study of the immature worm (Fig. 2) shows a transition stage in which these vitelline elements have assumed definite lobulations but have not yet become discrete. Fine ducts connect them with the common vitelline duct which leads into the ootype from the anterior aspect.

The ootype is surrounded by an encircling mass of glands. The naked ovum constitutes a very small part of the egg. The greater portion of the latter consists of yolk cells. The uterus is an uncoiled tube leading from the ootype directly to the genital atrium. I have not observed more than two eggs in the uterus while most of my specimens contain only one. The uterine egg measures about $100 \times 65\ \mu$, which measurements differentiate it from the two described species.

DISCUSSION

In an examination of thirty-two domestic ducks in Peking, Changsha, Wuchang and Kuling I have found no members of the genus Cyathocotyle and only one of the genus Strigea. There is still need of evidence, then, to show that the Chinese domestic duck is the natural host of *Cyathocotyle orientalis*. It does seem clear, however, that this duck can harbor the parasite until maturity and that the parasite when lodged in the cecum of the duck acts as a pathogene. While these experimental data are important in showing the relationship of parasite to larval and definitive host and the method by which infection of the definitive host may be accomplished, they are considerably more significant in demonstrating the relationship of the genus Cyathocotyle to the holostome group. Ward (1918: 409) refers to this genus as being "without differentiated regions," a statement which is true in the light of our usual conception of the holostomes, where there is ordinarily found a posterior region conspiculously set off from the anterior portion, the former containing the full complement of reproductive organs.

Odhner (1913) has shown that Cyathocotyle is fundamentally a holostome, although in many ways it partakes of distome characters. A comparison of the tetracotyle larva (Fig. 1), the transitional stage (Fig. 2) and the adult (Fig. 3) of *Cyathocotyle orientalis* indicates how many of the superficial characters may be modified in development from larva to adult. These changes are particularly embodied in the ventral suctorial apparatus. In the tetracotyle there is the normal complement of tetracotyliform suctorial structures. In addition to the oral sucker there are the lateral suctorial grooves, the hood and the functional ventral sucker, back of which is a typical set of paired anterior and single posterior suctorial lips. Careful inspection shows that the lateral suctorial grooves and the hood of the ventral pouch are merely the simplest kind of invaginations of the integument and superficial musculature and are not provided with special sphincter muscles, such as, for example, the lateral suctorial grooves of *Tetracotyle flabelliformis* (Faust 1918). In the transition to the mature worm these lateral depressions entirely disappear, which condition obtains even in the immature form as shown in fig. 2. As for the hood of the

pouch, it, too, becomes less conspicuous, while the posterior suctorial labia become resorbed. These latter changes are accompanied by a considerable enlargement of the functional ventral sucker, which comes to occupy the entire region of the ventral suctorial pouch, taking upon itself the structure and function of a powerful muscular sucking cup. In the immature worm the vestige of the ventral pouch is still apparent, but in the adult (Fig. 3) it has entirely atrophied. A most unique phenomenon has, therefore, resulted, namely, the complete disappearance of the original (posterior) ventral suctorial apparatus and the assumption of this structure and function by the anterior organ which is phylogenetically the genital pore (Faust 1919: 72).

After studying the literature on the described tetracotyliform larvae, I believe the data are too meager and the characters too poorly defined to predict genetic connections between larvae and adults on the basis of described characters. It seems, clear, however, that the larval group designated as "tetracotyles" belongs to several genera, among which are Cyathocotyle, Strigea, and probably Prohemistomum. While the usual method of transfer of the holostome larva is from mollusk directly to definitive host, intercalated secondary larval hosts are known, as for example in the case of *Tetracotyle pipientis,* found encysted in *Rana pipiens* (Faust 1918: 64) and many diplostomula, where the larvae become encysted in the derma and somatic muscles of fish.*

SUMMARY

1. A larval holostome, *Tetracotyle orientalis,* nov. spec. is described from Peking, China.

2. Its excretory system consists of two parts, a network of canals (the reserve bladder) and the system of tubules, capillaries, and flame-cells.

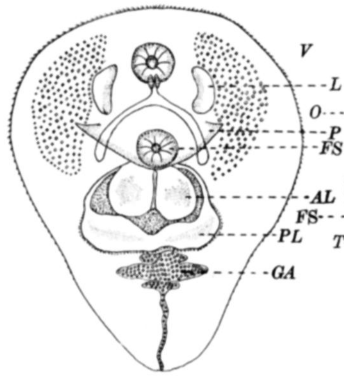
3. The excretory system shows five main tubules on each side of the body, each tubule draining a system of 32 dorsal and 32 ventral capillaries and flame-cells; each of these systems is based on a 5-fold dichotomy.

4. A young duck used for experimental feeding yielded at autopsy 75 holostomes. The controls were all negative.

5. Both immature and adult worms, designated as *Cyathocotyle orientalis* nov. spec., were recovered from the experimental host.

6. Comparison of tetracotyle, immature and adult worm indicates changes involved in the growth of the parasite and relationship of the genus and of the group.

* After this paper was finished an examination of the intestine of a mallard duck, *Anas boschas,* revealed the presence of 268 individuals of *Cyathocotyle orientalis* in the region of the small intestine and cecum. This is particularly significant in view of the fact that the Chinese domestic duck in which the worms were experimentally grown has been bred from the mallard duck.



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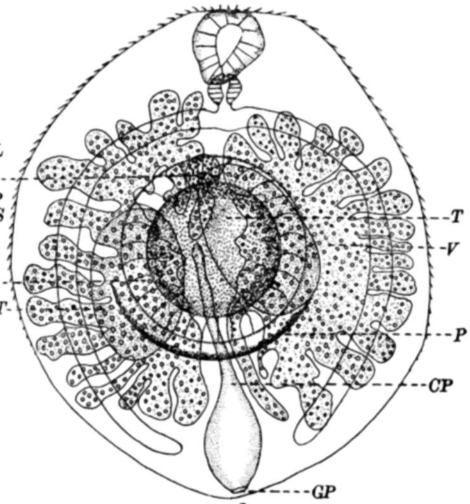

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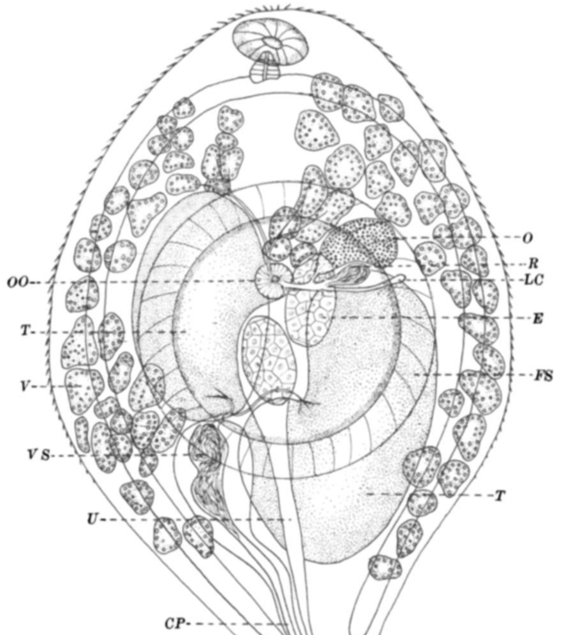

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PLATE IX

Fig. 1.—Ventral view of Tetracotyle larva of *Cyathocotyle orientalis,* showing larval organs and beginnings of genital system. × 100.

Fig. 2.—Dorsal view of immature *Cyathocotyle orientalis,* showing vestigial suctorial cup and development of genital organs. × 100.

Fig. 3.—Ventral view of mature *C. orientalis,* showing distribution of adult organs. × 80.

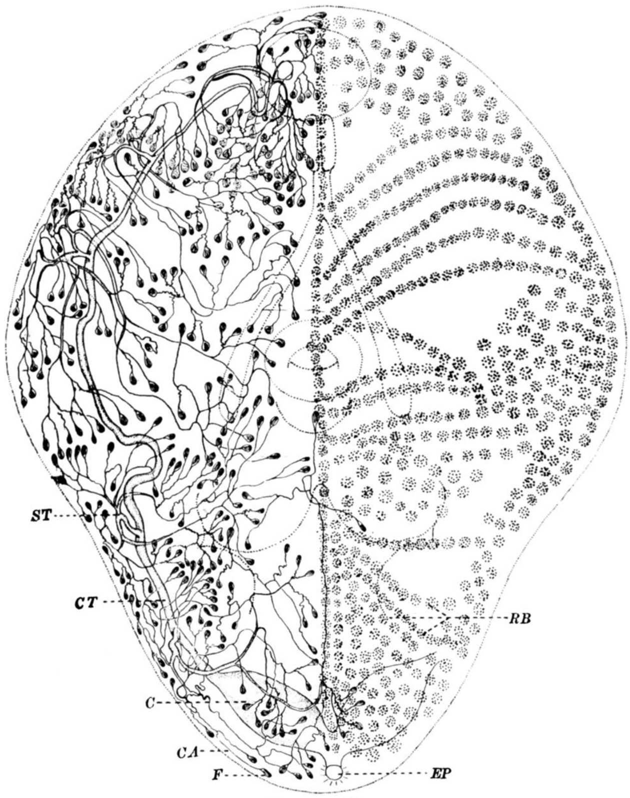

PLATE X

Fig. 4.—Dorsal view of Tetracotyle of *C. orientalis,* showing excretory system: right half, reservoir bladder; left half, tubules, capillaries and flame-cells. × 240.

ABBREVIATIONS

AL—Anterior suctorial lip
C—Cornu of bladder
CA—Capillary
CT—Collecting tubule
E—Uterine egg
EP—Excretory pore
F—Flame cell
FS—Functional ventral sucker
GA—Genital organs
GP—Genital pore
L—Lateral suctorial cup
LC—Laurer's canal
O—Ovary
OO—Ootype
P—Ventral suctorial pocket
PL—Posterior suctorial lip
R—Receptaculum seminis
RB—Reserve excretory bladder
ST—Secondary excretory tubule
T—Testes
U—Uterus
V—Vitelline follicle
VS—Vesicula seminalis

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